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**(54) Solvent dried polysaccharide sponges**

Lösungsmittel-getrocknete Polysaccharidschwämme

Eponges de polysaccharide séchées au moyen d'un solvant

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## Description

[0001] This invention relates to polysaccharide sponges, and to methods of making such sponges. Polysaccharide sponges made according to the method of the present invention are suitable for use as wound dressing materials.

[0002] Wound dressings comprising collagen sponges are commercially available under the Registered Trade Mark Fibracol. These materials are made by dispersing collagen in an aqueous medium, and then freeze drying the dispersion in order to remove water.

[0003] US-A-3157524 describes a method of making collagen sponge materials comprising the steps of: forming an aqueous dispersion of collagen; freezing the dispersion; treating the frozen dispersion in a series of anhydrous isopropanol baths to exchange isopropanol for the water in the frozen dispersion; followed by drying. There is no disclosure of preparing polysaccharide sponges by this method.

[0004] Wound dressings comprising polysaccharides, and in particular alginates, in the form of fibrous mats, fibrous ropes, aqueous gels or films are known. For example, alginate-based wound dressing pads are available under the Registered Trade Marks Kaltostat and Sorbsan. Alginates are particularly useful as wound dressing materials, because of their well-established haemostatic properties and high biological acceptability.

[0005] The known alginate dressings use calcium alginate or mixed calcium/sodium alginates. Calcium alginate is insoluble in water, but sodium alginate is readily soluble. The solubility of an alginate wound dressing material can therefore be adjusted by appropriate control of the ratio of calcium:sodium ions.

[0006] A soft, conformable polysaccharide sponge can be prepared by freeze drying a solution of a soluble polysaccharide such as sodium alginate. However, such a sponge is of limited value as a wound dressing material, because of its solubility in wound exudate.

[0007] Freeze drying a dispersion of calcium alginate in water also leads to a sponge which is unsuitable for use as a wound dressing material, because it is extremely friable.

[0008] The present invention is based on the surprising discovery that a polysaccharide sponge which is prepared by treatment of a frozen aqueous solution of soluble polysaccharide with a cross-linking agent dispersed in a hygroscopic non-aqueous solvent followed by drying is soft and highly conformable with minimal tendency to shed particles or fibres in use. It is therefore well suited for use as a wound dressing material.

[0009] The present invention provides a method for preparing a polysaccharide sponge, comprising the steps of: (a) providing a solution of soluble polysaccharide in water; (b) freezing the solution to form a frozen solution; (c) immersing the frozen solution in a water-miscible organic solvent containing a cross-linking agent to exchange said solvent for at least a part of the

water in the frozen solution while cross-linking the polysaccharide to render it water insoluble; and (d) drying the resulting cross-linked and solvent-exchanged polysaccharide material.

[0010] The soluble polysaccharide used in step (a) is preferably selected from the group consisting of soluble alginates such as sodium alginate, xanthan gum, guar gum, locust bean gum, chitosan, carboxymethyl cellulose salts, hydroxyethyl cellulose, hyaluronic acid, pectin, and mixtures thereof.

[0011] Preferably, the soluble polysaccharide (and hence the final polysaccharide sponge product) comprises at least 25% by weight of alginate, more preferably at least 75% by weight of alginate, and most preferably it consists essentially of alginate.

[0012] The cross-linking agent used in step (c) may be any ionic or covalent cross-linking agent. Preferred cross-linking agents are selected from the group consisting of glutaraldehyde, dicyclohexyl carbodiimide, hexamethylene diisocyanate, salts of multivalent cations, salts of multivalent anions, and mixtures thereof. The particular cross-linking agent used will depend on the nature of the soluble polysaccharide. For example, soluble alginates such as sodium alginate are preferably cross-linked by the addition of multivalent cations, such as divalent or trivalent metal cations in the form of salts, in particular calcium chloride or zinc chloride. These chlorides are soluble in organic solvents. On the other hand, guar gum is preferably cross-linked by reaction with polyanions, especially borate or phosphate anions. Likewise, chitosan can be cross-linked by reaction with borate or phosphate ions, such as sodium tripolyphosphate or sodium tetraborate.

[0013] The present invention also provides a substantially water-insoluble polysaccharide sponge obtainable by a method according to the invention. The present invention further provides a wound dressing or implant comprising such a sponge.

[0014] Typically, the polysaccharide will be dissolved in the aqueous medium in an amount ranging from 0.2% to 5% w/v, and more preferably from 0.5% to 3% w/v. For example, the solution used in step (a) may contain polysaccharide in an amount of approximately 1% w/v.

[0015] The key step in the process according to the present invention is the solvent exchange and cross-linking step (c). It has been found that exchanging the water in the frozen polysaccharide solutions by a volatile solvent at the same time as cross-linking the polysaccharide to render it insoluble, followed by drying under relatively mild conditions, results in the formation of polysaccharide sponges with superior physical properties. The resulting sponges are softer, more conformable and less friable than polysaccharide sponges produced by freeze drying insoluble polysaccharide suspensions. Furthermore, the methods according to the present invention are faster, less energy-intensive, and more suited to continuous production than freeze-drying.

[0016] Preferably, the organic solvent is a hygroscopic, volatile organic solvent such as a substantially anhydrous C<sub>1</sub>-C<sub>5</sub> monohydric alcohol or a C<sub>3</sub>-C<sub>6</sub> ketone, or mixtures thereof. Preferred solvents include ethanol, propanol, isopropanol, n-butanol, isobutanol, t-butyl alcohol, amyl alcohol and acetone. Most preferred is anhydrous isopropanol.

[0017] The solvent baths may be at any temperature at which the organic solvent is liquid, but they are preferably at or near ambient temperature (0-30°C). This results in solvent exchange and cross-linking taking place simultaneously with thawing of the frozen polysaccharide dispersion.

[0018] Preferably, the solvent exchange step is carried out in two or more successive baths of the organic solvent. The second and subsequent solvent baths may also contain the cross-linking agents. The use of multiple solvent baths results in more complete exchange of the organic solvent for water in the polysaccharide material, thereby reducing drying time and energy, and giving a product with better physical properties.

[0019] Typically, the volume of each bath of organic solvent used to wash 1 litre of frozen polysaccharide solution or suspension will be from 0.5 to 10 litres, preferably from 1 to 5 litres. Following the washing step, it will usually be appropriate to dry the alginate sponge in air at ambient temperature up to 80°C or under reduced pressure, optionally with a preliminary squeezing or blotting step to remove excess solvent.

[0020] When a multivalent anion or cation salt is used as the cross-linking agent in the organic solvent, it is preferably present in an amount from 0.1 to 50% w/v, and more preferably from 1% to 20% w/v. The preferred salts are anhydrous calcium chloride and sodium tetraborate. Anhydrous zinc chloride and sodium tripolyphosphate may also be used.

[0021] The polysaccharide used in the method of the present invention may be modified prior to forming the sponge. For example, the polysaccharide may be partially crosslinked using conventional crosslinking agents, or it may be combined with other biopolymers which are appropriate for use in wound dressing materials. For example, mixtures of alginate with chitosan, xanthan gum, guar gum or pectin may be used.

[0022] Specific embodiments of the present invention will now be described further, by way of example, as follows:

#### Example 1

[0023] A 10ml sample of a solution of sodium alginate (LVG 008 281 05, molecular weight 200,000-250,000, mannuronic acid/guluronic acid ratio of 0.45 supplied by Pronova Biopolymer, Drammen, Norway) at a concentration of 1% w/v in deionised water is blast frozen at -30°C for 30 minutes. The frozen block is immersed in 20ml of anhydrous isopropanol containing 10% w/v of anhydrous CaCl<sub>2</sub> and shaken for 2 hours at 25°C until

defrosted. The resulting material is removed from the isopropanol bath, blotted with absorbent tissue, and then dried in air at 60°C overnight. The resulting material is a white, soft, flexible sponge having low friability, and low solubility in water.

#### Example 2

[0024] The procedure of Example 1 is repeated, but with the final drying step carried out under vacuum at 25°C overnight. A substantially similar product is obtained as in Example 1.

#### Example 3

[0025] The procedure of Example 1 is repeated, but with a second solvent treatment step prior to the final drying step. The second solvent treatment step is carried out in a second 20ml bath of fresh anhydrous isopropanol containing 20% w/v anhydrous CaCl<sub>2</sub>. A substantially similar product is obtained as in Examples 1 and 2.

#### Example 4

[0026] The procedure of Example 3 is repeated, but with no CaCl<sub>2</sub> present in the second 20ml bath of fresh anhydrous isopropanol. A substantially similar product is obtained as in Examples 1-3.

#### Example 5

[0027] The procedure of Example 3 is repeated, but with a third solvent treatment step prior to the drying step. The third solvent treatment step is carried out in a third 20ml bath of fresh anhydrous isopropanol containing 20% w/v CaCl<sub>2</sub>. A product substantially similar to those in the previous Examples is obtained.

#### Example 6

[0028] The procedure of Example 5 is repeated, but with no CaCl<sub>2</sub> present in the third solvent bath. A product substantially similar to those in the previous examples is obtained.

#### Example 7

[0029] The procedure of Example 1 is repeated, but with replacement of the 1% alginate solution by an aqueous solution containing 0.5% w/v alginate and 0.5% w/v chitosan. The solvent exchange bath contains 1% w/v CaCl<sub>2</sub> in anhydrous isopropanol. The resulting material is soft, flexible and absorbent.

#### Example 8 (Comparative Example)

[0030] A 10ml sample of a suspension of 1% w/v cal-

cium alginate fibers in water is blast frozen at -30°C for 30 minutes.

[0031] The frozen suspension is freeze-dried overnight at -20°C to 25°C. The resulting material is soft, but friable. It breaks when bent and sheds fibers to the touch.

#### Example 9

[0032] The procedure of Example 1 is repeated, but with replacement of the sodium alginate by guar gum, and with replacement of the calcium chloride cross-linking agent by sodium tetraborate. The product is a soft, conformable, insoluble sponge.

#### Example 10

[0033] The procedure of Example 9 is repeated, but with replacement of the guar gum by a mixture of 50% w/w guar gum and 50% w/w xanthan gum. A similar product to that of Example 9 is obtained.

#### Example 11

[0034] The procedure of Example 10 is repeated, but with replacement of the sodium tetraborate cross-linking agent by calcium chloride, as in Example 1. A similar product to that of Example 10 is obtained.

#### Example 12

[0035] The integrity of the sponges prepared as above was assessed as follows: A square (1.5 x 1.5 cm) of each sponge sample was added to 10 ml of culture medium (Dulbecco's modification of Eagles medium supplemented with 10% foetal calf serum) in a 30 ml Sterilin® container. Initial observations were made, and the samples were then incubated at 37°C. Further observations were made at 6, 22 and 174 hours.

[0036] It was found that the solvent dried, cross-linked samples prepared as specified in Example 1 swelled in the serum at ambient temperature, but remained intact. After 7 days, one or two small fibers of the material were visible in the solution, but the integrity of the bulk material was substantially maintained.

[0037] In contrast, a sodium alginate sponge prepared by freeze drying without any cross-linking step was completely dissolved in the solution after 7 hours. A calcium alginate sponge produced by freeze drying a dispersion of calcium alginate as described in Example 8 started to disintegrate as soon as it was placed in the solution. After 20 hours several large fragments of the sponge were floating in the solution, and after 72 hours there were many free calcium alginate fibers in the solution.

#### Example 13

[0038] The absorbency of the sponges prepared according to the present invention is evaluated as follows.

[0039] A number of samples of sponge (50:50 chitosan:alginate cross-linked with calcium chloride) prepared by the method of Example 7 were weighed, immersed in phosphate buffered saline solution at 37°C for 30 minutes and then weighed again. The liquid absorption due to immersion was found to increase the weight of the samples by a factor of  $21.7 \pm 3.5$ .

[0040] A number of comparative samples of sponge (50:50 chitosan:calcium alginate) prepared by a process analogous to that in Example 8 were similarly tested. The liquid absorption due to the immersion was found to increase the weight of the samples by a factor of  $14.2 \pm 3.7$ .

[0041] The above examples are intended for the purpose of illustration the scope of the accompanying claims will be apparent to the skilled reader.

#### Claims

1. A method of preparing a polysaccharide sponge, comprising the steps of:
  - (a) providing a solution of a soluble polysaccharide in water;
  - (b) freezing the solution to form a frozen solution;
  - (c) immersing the frozen solution in a water-miscible organic solvent containing a cross-linking agent to exchange said solvent for at least a part of the water in the frozen solution while cross-linking the polysaccharide to render it water insoluble; and
  - (d) drying the resulting cross-linked and solvent-exchanged polysaccharide material.
2. A method according to claim 1 wherein the soluble polysaccharide is selected from the group consisting of soluble alginates, xanthan gum, guar gum, chitosan, carboxymethyl cellulose, hydroxymethyl cellulose, hyaluronic acid, pectin and mixtures thereof.
3. A method according to claim 1 or 2, wherein the soluble polysaccharide comprises at least 25% by weight of one or more alginates.
4. A method according to claim 3, wherein the soluble polysaccharide comprises at least 75% by weight of one or more alginates.
5. A method according to any preceding claim, wherein the cross-linking agent is selected from the group consisting of glutaraldehyde, dicyclohexyl carbodi-

- imide, hexamethylene diisocyanate, salts of multivalent cations and salts of multivalent anions.
6. A method according to claim 5, wherein the cross-linking agent is a salt of a divalent or trivalent metal cation. 5
  7. A method according to claim 6, wherein the cross-linking agent is calcium chloride or zinc chloride. 10
  8. A method according to claim 5, wherein the cross-linking agent comprises a borate or a phosphate. 15
  9. A method according to any preceding claim, wherein the solvent-exchanged polysaccharide product of step (c) is immersed in at least one further bath of water-miscible organic solvent to effect more complete exchange of organic solvent for water in said polysaccharide material prior to the drying step (d). 20
  10. A method according to any preceding claim, wherein the water-miscible organic solvent comprises a C<sub>1</sub>-C<sub>4</sub> monohydric alcohol or a C<sub>3</sub>-C<sub>6</sub> ketone. 25
  11. A method according to any preceding claim, wherein said drying step (d) is carried out under sub-atmospheric pressure. 30
  12. A substantially water-insoluble polysaccharide sponge obtainable by a method according to any preceding claim. 35
  13. A wound dressing or implant comprising a substantially water insoluble polysaccharide sponge according to claim 12. 40
- lysaccharid ausgewählt ist aus der Gruppe bestehend aus löslichen Alginaten, Xanthangummi, Guar gummi, Chitosan, Carboxymethylcellulose, Hydroxymethylcellulose, Hyaluronsäure, Pektin und Gemischen davon.
3. Verfahren nach Anspruch 1 oder 2, wobei das lösliche Polysaccharid mindestens 25 Gewichtsprozent von einem oder mehreren Alginaten umfaßt.
  4. Verfahren nach Anspruch 3, wobei das lösliche Polysaccharid mindestens 75 Gewichtsprozent von einem oder mehreren Alginaten umfaßt.
  5. Verfahren nach jedem voranstehenden Anspruch, wobei das kreuzvernetzende Mittel ausgewählt ist aus der Gruppe bestehend aus Glutaraldehyd, Dicyclohexylcarbodiimid, Hexamethylen-diisocyanat, Salzen von multivalenten Kationen und Salzen von multivalenten Anionen.
  6. Verfahren nach Anspruch 5, wobei das kreuzvernetzende Mittel ein Salz eines divalenten oder trivalenten Metallkations ist.
  7. Verfahren nach Anspruch 6, wobei das kreuzvernetzende Mittel Kalziumchlorid oder Zinkchlorid ist.
  8. Verfahren nach Anspruch 5, wobei das kreuzvernetzende Mittel ein Borat oder ein Phosphat umfaßt.
  9. Verfahren nach jedem voranstehenden Anspruch, wobei das Lösemittel-ausgetauschte Polysaccharidprodukt von Schritt (c) in mindestens ein weiteres Bad von Wassermischbarem organischem Lösemittel eingetaucht wird, um einen vollständigen Austausch von organischem Lösemittel für Wasser in dem Polysaccharidmaterial vor dem Trocknungsschritt (d) zu bewirken.
  10. Verfahren nach jedem voranstehenden Anspruch, wobei das Wasser-mischbare organische Lösemittel ein C<sub>1</sub>-C<sub>4</sub> monohydrischer Alkohol oder ein C<sub>3</sub>-C<sub>6</sub> Keton umfaßt.
  11. Verfahren nach jedem voranstehenden Anspruch, wobei der Trocknungsschritt (d) unter sub-atmosphärischem Druck durchgeführt wird.
  12. Im wesentlichen Wasser-unlöslicher Polysaccharidschwamm, erhältlich durch ein Verfahren nach einem voranstehenden Anspruch.
  13. Wundverband oder Implantat, umfassend einen im wesentlichen Wasser-unlöslichen Polysaccharidschwamm nach Anspruch 12.

#### Patentansprüche

1. Verfahren zur Herstellung eines Polysaccharidschwamms, umfassend die Schritte von: 40
  - a) zur Verfügungstellen einer Lösung eines löslichen Polysaccharids im Wasser;
  - b) Frieren der Lösung, um eine gefrorene Lösung zu bilden; 45
  - c) Eintauchen der gefrorenen Lösung in ein Wasser-mischbares organisches Lösemittel, das ein kreuzvernetzendes Mittel enthält, um das Lösemittel für mindestens einen Teil des Wassers in der gefrorenen Lösung während Kreuzvernetzen des Polysaccharids auszutauschen, um es wasserunlöslich zu machen; und 50
  - d) Trocknen des erhaltenen kreuzvernetzten und Lösemittel-ausgetauschten Polysaccharidmaterials. 55
2. Verfahren nach Anspruch 1, wobei das lösliche Po-

## Revendications

1. Procédé de préparation d'une éponge de polysaccharide comprenant les étapes de :
  - (a) fournir une solution de polysaccharide soluble dans l'eau ; 5
  - (b) geler la solution pour former une solution gelée ; 10
  - (c) immerger la solution gelée dans un solvant organique miscible dans l'eau contenant un agent de réticulation, pour remplacer au moins une partie de l'eau par ledit solvant dans la solution gelée, tout en réticulant le polysaccharide pour le rendre insoluble dans l'eau ; et 15
  - (d) sécher le matériau de polysaccharide obtenu réticulé et à solvant échangé. 20
2. Procédé selon la revendication 1, dans lequel le polysaccharide soluble est choisi dans le groupe constitué des alginates solubles, la gomme de xanthane, la gomme de guar, le chitosane, la carboxyméthylcellulose, l'hydroxyméthylcellulose, l'acide hyaluronique, la pectine et des mélanges de ceux-ci. 25
3. Procédé selon les revendications 1 ou 2, dans lequel le polysaccharide soluble contient au moins 25% en poids d'un ou plusieurs alginates. 30
4. Procédé selon la revendication 3, dans lequel le polysaccharide soluble contient au moins 75% en poids d'un ou plusieurs alginates. 35
5. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'agent de réticulation est choisi dans le groupe constitué du glutaraldéhyde, de la dicyclohexylcarbodiimide, du diisocyanate d'hexaméthylène, des sels de cations multivalents et des sels d'anions multivalents. 40
6. Procédé selon la revendication 5, dans lequel l'agent de réticulation est un sel de cation métallique divalent ou trivalent. 45
7. Procédé selon la revendication 6, dans lequel l'agent de réticulation est le chlorure de calcium ou le chlorure de zinc. 50
8. Procédé selon la revendication 5, dans lequel l'agent de réticulation contient un borate ou un phosphate. 55
9. Procédé selon l'une quelconque des revendications précédentes, dans lequel le produit de polysaccharide à solvant échangé de l'étape (c) est immergé dans au moins un autre bain de solvant organique miscible dans l'eau pour effectuer un échange plus complet de l'eau par le solvant organique dans ledit matériau de polysaccharide avant l'étape (d) de séchage.
10. Procédé selon l'une quelconque des revendications précédentes, dans lequel le solvant organique miscible dans l'eau contient un alcool monohydrique C<sub>1</sub>-C<sub>4</sub> ou une cétone C<sub>3</sub>-C<sub>6</sub>.
11. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'étape (d) de séchage est effectuée sous pression sub-atmosphérique.
12. Éponge de polysaccharide substantiellement insoluble dans l'eau susceptible d'être obtenue par un procédé selon l'une quelconque des revendications précédentes.
13. Pansement ou implant contenant une éponge de polysaccharide substantiellement insoluble dans l'eau selon la revendication 12.